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WHYON: Experiments on the Behaviour of Leishmania &
Allied Flagellates in Bugs & Fleas &c.

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EXPERIMENTS ON THE BEHAVIOUR OF LEISHMANIA AND ALLIED FLAGELLATES IN BUGS AND FLEAS, WITH SOME REMARKS ON PREVIOUS WORK.

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IT was first shown by Patton that the parasite of Indian Kala Azar would develop into flagellates in the gut of *Cimex rotundatus*. More recently Patton has repeated these experiments not only with *Cimex rotundatus* but also with *Cimex lectularius*. In the latter series of experiments he found that the flagellate forms first produced give rise ultimately to small post-flagellate forms which have no flagellum. It is these post-flagellate forms which Patton thinks enter the body when the infected bug bites. In this manner, probably by a regurgitation from the stomach of the bug during its feed, the post-flagellate forms give rise to Kala Azar. The direct transmission of Kala Azar to animals by the bug has not been done.

In 1910 I discovered that the bed-bug of Bagdad (*Cimex rotundatus*?) would take up leishmania from Oriental Sore and that in it the parasites developed into flagellate forms. This observation was later confirmed by Patton, who was working with Oriental Sore in Cambay in India. Patton came to the conclusion that the bug was also the transmitter of Oriental Sore. In my report on Oriental Sore I contested the view that the bed-bug was the transmitter of Oriental Sore and Kala Azar, and explained the development which took place in the bug as comparable to that which the leishmania would undergo when introduced into blood agar media in the test-tube.

Will Leishmania tropica develop in Stegomyia fasciata?

In his last report on the development of the parasite of Indian Kala Azar in the bug Patton points out that my view cannot be the right one, since he has shown that various other blood-sucking arthropoda which take up a large quantity of blood with the leishmania do not permit of these parasites developing to the flagellate stage. The arthropoda mentioned are *Culex fatigans*, *Neocellia stephensi*, *Stegomyia sguens*, *Ornithodoros savignyi*, *Pediculus capitis*, and *Pediculus vestimenti*. It is also pointed out that the parasite of Cambay boil will not develop in *Stegomyia*. In Bagdad I conducted experiments with *Stegomyia fasciata*, and found that about 10 per cent. of such *Stegomyia* which had fed upon the sore were found to have taken up leishmania when dissected immediately afterwards; and in six of these mosquitoes out of eighty which had fed upon the sore and were dissected at intervals after feeding, round and flagellate forms were seen which I concluded were most probably developmental forms of leishmania; while of over

seventy *Stegomyia* which had fed upon a healthy person and were dissected as controls, none were found to harbour any flagellates. It will be seen that the percentage of mosquitoes which had taken up parasites is about equal to the percentage of those found infected when dissected some time after feeding. This in itself is great presumptive evidence in favour of the flagellates seen in the *Stegomyia* being in reality developmental forms of leishmania. Now Patton doubts very much whether the flagellates seen by me were flagellate forms of leishmania, chiefly because he obtained no evidence of such a development with the *Stegomyia* in Cambay. It is stated that a large number were fed on the case, some on the margin of the sore and some on distant parts. We are not told how many mosquitoes were used, nor if by immediate dissection after feeding any of the *Stegomyia* were proved to have taken up parasites. Unless it is known that the *Stegomyia* can take up parasites when feeding in the way adopted for experiment, it is useless to state that the leishmania will not develop into flagellates. Patton states that he was unable to find any parasites changed or unchanged in his mosquitoes, so that he has no evidence whatever that the *Stegomyia* had taken up parasites when feeding. His method of feeding the *Stegomyia*, either because the case was not a good one or else the mosquitoes fed too far away from the lesion, was evidently not one which would enable the mosquitoes to take up leishmania. In my experiments the mosquitoes fed on top of a non-ulcerating sore, and, as I have stated, at least 10 per cent. of these took up parasites directly from the sore; and when one finds that about 10 per cent. of *Stegomyia* fed upon the sore and dissected after varying intervals show forms which resemble developmental forms of leishmania, the most reasonable conclusion is that they are really such, especially when an equal number of *Stegomyia* fed upon a healthy person gave negative results when dissected after similar intervals. The fact that the larva of *Stegomyia* may contain a flagellate does not really affect the issue, since it was shown that this did not appear in any of the control mosquitoes dissected. Patton does not tell us if the larva of the Cambay *Stegomyia* may harbour such a flagellate.

The Gut of the Bed-bug as a Culture-tube for Flagellates.

Now Patton's failure to obtain a development of the *Leishmania donovani* in the arthropoda mentioned above and also in *Conorrhinus rubrofasciatus* is in itself no proof that the leishmania develop in the bed-bug in any other way than they do in the blood agar medium. Though leishmania will not flagellate in these arthropoda, it is still possible that they develop in the bug as they do in the culture-tube. It may simply mean that these arthropoda have some substance in the gut which is injurious to the leishmania (this must be so), while the gut-contents of the bug has no such substance. As a matter of fact, there is considerable evidence of the innocuous nature of the gut contents of the bug.

1. I have shown that the spirochætes of relapsing fever when taken up by the bug will remain active for as much as seven days and appear normal in stained films after three weeks in the bug's gut*.

2. It has recently been shown by Brumpt that *Schizotrypanum cruzi*,

* See Graham V. Smith, "On some Cases of Relapsing Fever in Egypt and the Question of Carriage by Domestic Vermin," p. 36. John Bale, Sons, & Danielsson, Ltd., London.

whose natural host is a species of *Conorrhinus*, will undergo a development in the bed-bug, and that the forms which are found both in the mid and hind gut are infective for ten days when injected into animals. Not only does *Schizotrypanum cruzi* develop in *Cimex lectularius* but also in *Cimex boueti* and *Ornithodoros moubata*, which are all unable to transmit the infection as does the true host of the *Schizotrypanum*.

3. It was shown by Swellingrebel and Strickland that *Trypanosoma lewisi* will undergo a development in *Cimex lectularius*, and that as late as nine days crithidial forms occur in the mid gut. I have repeated this observation and have found that on the eleventh day trypanosome, crithidial, herpetomonas, and leishmania forms occur. Some of the trypanosomes bear a striking resemblance to the small infective forms which appear in the hind gut of infected fleas. It is hardly necessary to say that the bug is not the host of *T. lewisi*.

4. I have fed bugs on rats infected with *Trypanosoma rhodesiense*, and after seven days not only can trypanosome and crithidial forms be found, but also many rounded parasites. The trypanosomes, however, disappear very rapidly from the intestines of fleas.

It is thus quite clear that the gut of the bug when it contains blood acts as a culture-tube in which various organisms can live and develop. This must depend to a great extent on the absence of any harmful material which might destroy them. It is hardly to be wondered at that such an easily cultured organism as the leishmania should also undergo a development in the bug's gut. Since we know that other flagellates will develop here, it would be a remarkable fact if the leishmania did not so develop. As a matter of fact, the persistence of the flagellates in the gut of the bug appears to be in proportion to the ease with which they are to be cultured in the blood agar medium. Hence the fact that the leishmania of Kala Azar and Oriental Sore become flagellates in the bug's gut is in itself no proof whatever that these insects are the transmitters of these diseases. Patton has stated that in his opinion the parasite of Mediterranean Infantile Kala Azar will be found to flagellate in *Cimex lectularius*. I will extend this prophesy and include the leishmania of Canine Kala Azar and of Dermal Leishmaniasis of South America.

The Final Stage of Development of Leishmania in the Bed-bug.

In his last report on the parasite of Kala Azar, Patton lays great emphasis on the appearance in the bugs of what he calls the post-flagellate forms, which are rounded forms developed from the flagellate forms. In the life-history of a typical *Herpetomonas* of any insect such rounded forms are developed from the flagellates in the hind gut, and it is these which, protected by some kind of covering, pass out in the faeces, and are responsible for the transmission of the infection to a new host. The round forms of leishmania which appear in the bugs after the eighth day are compared by Patton to the post-flagellate forms of a *Herpetomonas*, and on that account he is convinced that he has traced the whole development of the leishmania from the forms ingested through the flagellate stage to the post-flagellate. In the bug, however, the post-flagellate is not formed in the hind gut as is usually the case with the true *Herpetomonas* of insects, but in the mid gut, and it is supposed that they gain entrance to a new human host by regurgitation when the bug

bites. Whether the forms seen in the bug are true post-flagellate forms in the same sense as those that occur in insects must be an exceedingly difficult matter to decide. Patton, who has had much experience with the *Herpetomonas* of insects, says he can recognise them by their peculiar staining reaction. A staining reaction, especially by a Romanowsky stain and in such a variable medium as the gut contents of an insect, is hardly a reliable test. Further, it is known that in cultures of leishmania in blood agar there is first the production of a flagellate stage and ultimately the production of round forms without a flagellum. Row has insisted on the fact that these round forms are really post-flagellates; but whether this is so or not it is evident that the ultimate production of round forms in the mid gut of the bug is a further illustration of the similarity of the behaviour of the leishmania in the culture-tube and in the bug's intestine. Patton has shown that if the bug during the stage in which flagellate forms of leishmania are present in its gut has a second feed of blood, the flagellate forms are destroyed, and this he attributes to the presence of complement in the blood. It is this complement which explains in his mind the absence of flagellate forms in the body of man, and he claims that the round post-flagellate forms must be produced before infection can take place. It has been frequently shown that artificial cultures of leishmania may produce both Oriental Sore and Kala Azar when injected into animals. It must then be some more resistant form in these cultures—a post-flagellate—which causes the infection, for, according to Patton, the flagellate forms are destroyed by the blood. It thus appears that the development of the leishmania in the bug is a picture—and, as a matter of fact, a somewhat restricted picture—of the development undergone by leishmania in the blood agar media. Just as in the test-tube when the culture is old and nourishment is exhausted round forms appear, so in the bug the so-called post-flagellates are produced when the blood taken up by the bug is reaching the completion of its digestion.

The Development of Leishmania in the Bug is a very restricted one.

Another point which to my mind is a very strong argument against the bed-bug being the true host of these organisms is the very restricted development undergone by them in the bug. Experiments with Oriental Sore are much more difficult to conduct than with Kala Azar, in which comparatively large numbers of parasites may be present in the peripheral blood. When a bug feeds upon the sore one has no criterion as to how many parasites have been taken up, but in the case of the peripheral blood of Kala Azar cases it would be possible by measuring the quantity of blood taken up by a bug to estimate fairly accurately the number of parasites it had ingested. When, for instance, as many as 250 parasites occur in a blood-film, it must be certain that a bug feeding on the case had taken up a considerable number of parasites. Now the point I wish to emphasise is this: namely, that the extent to which the bug becomes infected—or, in other words, the number of flagellates to be found in its gut after a few days—varies directly with the number of parasites ingested. In experiments on Oriental Sore, where the number of leishmania taken up must be very small, one never finds a large infection with flagellates; while in Patton's last experiments, in which the bugs had fed upon blood from a heavily infected case of Kala Azar with numerous parasites in the peripheral blood, he obtained large numbers of flagellates. If the bug were the true host of the leishmania a complete

infection of the gut would take place whether many or few parasites had been ingested; the only difference would be that the bug would take a longer time to show a large infection when the parasites were few. In the case of insects which are infecting themselves with herpetomonas by the ingestion of post-flagellate forms voided by another, it is surely not necessary for the insect to take up large numbers of these in order to become heavily infected. Presumably the ingestion of one such post-flagellate form would be sufficient to give rise ultimately to the heavily infected gut so commonly seen in insects harbouring herpetomonas. If the bug were the true host of leishmania it would be expected that the ingestion of only a few parasites would be sufficient to produce a large infection of the gut.

The Effect of a Second Feed of Blood on the Flagellates in Insects.

Patton has shown that a second feed of blood is fatal to the flagellate forms of the leishmania which may have developed in the bug. This, again, appears to me to be an argument against the bug hypothesis. I have recently kept under observation fleas infected with herpetomonas and others infected with *Trypanosoma lewisi*. One flea has been observed for two months, during which time it has had sixteen feeds of human blood. By an examination of the faeces voided during the feeding act I have found that it has constantly remained infected with herpetomonas, which were passed in the faeces in very large numbers at every feed, so that a constant active multiplication must have gone on. Two fleas infected with *Trypanosoma lewisi* were similarly fed on human blood during the course of one month. The faeces constantly showed the small infective trypanosomes characteristic of *T. lewisi*. Why, then, did not the constant feeding destroy the flagellates in the guts of these animals? The explanation must be that the flagellates in question were in their true host and were accustomed to human blood. This should be true of the leishmania in its true host, and the disappearance of the herpetomonas forms when the bug has a second feed simply shows that the flagellate is in an unnatural environment. Patton has shown that the natural herpetomonas of *Culex fatigans* disappears from this insect when it has a feed of blood. This is not remarkable if we remember that the herpetomonas is really a flagellate of the larva of the mosquito, which is not a blood-sucker, and remains in the adult only so long as the intestinal contents approximate in composition to those of the larva. There is one observation which might be produced as an argument against this contention. Miss Robertson has shown that batches of *Glossina* fed upon cases of human trypanosomiasis show a greater percentage of individuals infected if they have had only a single feed of blood. The explanation of this is at present not clear, for we know that an infected *Glossina* may infect a series of animals at intervals of several days.

Patton sees in this effect of a second feed of blood an explanation of the tendency of Kala Azar to remain localised in Madras and Oriental Sore in Cambay, and says: "This puzzling fact in connection with the disease can now be better explained, when it is known that though many bugs may become infected, only those which do not feed again till the parasite has passed back to its post-flagellate stage are infective; the number capable of transmitting the parasite must then necessarily be reduced." There is in this assumption a fallacy of fundamental importance. In every case where the intermediate host is well known, it is not this which is the prime cause in

the spread of the disease outside any given locality. It is not, as a rule, the intermediate host which transports the infection from place to place, but the reservoir, which is frequently man himself, who passes from one locality where the disease exists to another where the necessary intermediate host already occurs. Trypanosomiasis spreads because infected individuals travel, and not because the *Glossina* spreads the infection; Yellow Fever is similarly spread rather by the moving about of infected persons than by the transportation of the infected *Stegomyia*; Plague is carried by the rats and Malaria by man; and in the case of Kala Azar there is nothing to prevent the infected individual moving from Madras to another town where bugs are ready to feed and become infected. In man the infection is of long duration, while there is no evidence to show that bugs remain infective for any lengthy period, even if we assume they are so at any time. It must constantly happen that people suffering from Kala Azar and Oriental Sore move from Madras and Cambay respectively to other localities where bugs abound. Why, then, do not these cases become the starting-point in the infection of some new locality? To explain this by assuming that the majority of bugs soon disinfect themselves by a second feed is to close one's eyes to the more important factor in the spread of disease outside any given area. It does sometimes happen that an infected intermediate host is transported from one place to another, but this is a method of spread of disease which is quite subsidiary to that brought about by the wanderings of man himself.

On the Nomenclature of the Group Leishmania.

On the subject of the nomenclature of the parasites of Kala Azar and Oriental Sore, Patton is of opinion that as they conform to the herpetomonas type they should be known by the generic name of *Herpetomonas* and not *Leishmania*, and he makes the somewhat remarkable statement that "as the parasite of Sleeping Sickness passes one stage of its life-history in the blood and organs of man, and its multiplicative stage in an invertebrate *Glossina palpalis*, so in the same way the parasite of Indian Kala Azar passes one stage of its life-history in the blood and organs of man and its multiplicative stage in an invertebrate *Cimex rotundatus*; there would, however, be no justification for placing the parasite of Sleeping Sickness in any other genus but *Trypanosoma*." Now it is this very reason which, justifying the inclusion of the parasite of Sleeping Sickness in the genus *Trypanosoma*, supports the creation of a new genus *Leishmania*. The genus *Trypanosoma* includes flagellates which have both a vertebrate and invertebrate host, and the parasite of Kala Azar agrees with it in this; but the genus *Herpetomonas* includes flagellates which have only one, and that an invertebrate host. The fact that the parasite of Kala Azar has two hosts and can live and multiply in the organs of a vertebrate shows it to be profoundly different from the true *Herpetomonas*, which lives only in the invertebrate. This fact alone would be sufficient to establish the genus *Leishmania*. It is admitted that the *Leishmania* resemble the *Herpetomonas* in their life-histories as far as we know them, but in any classification transition forms between the groups occur, as must be so from an evolution standpoint. When a *Herpetomonas* becomes so changed that it has acquired the power of living and developing as an intracellular parasite in the body of a warm-blooded animal, and giving rise to such a serious disease as Kala Azar, we are justified in concluding that it has passed out of the genus *Herpetomonas*, from which it originated, into the genus *Leishmania*.

EXPERIMENTS ON THE DEVELOPMENT OF *LEISHMANIA TROPICA*
IN *CIMEX LECTULARIUS*.

In an earlier note I pointed out that I had produced in myself an Oriental Sore by inoculation, performed in Aleppo in August 1911. Six and a half months later the sores—three in number—appeared. They have increased in size, are of the non-ulcerating variety, and admirably suited for feeding experiments. Numbers of bed-bugs, *Cimex lectularius*, nearly all of which were hatched in the laboratory, were fed on the sore and kept in the cool incubator at 22°-23° C. for varying intervals before dissection. The conditions under which the experiments were conducted were ideal ones for giving good results. I had formerly shown that in Bagdad the *Leishmania tropica* would develop into flagellates in the bug (*Cimex rotundatus*?).

I have now obtained proof that a similar development to flagellate forms will take place in *Cimex lectularius*. The development has been most disappointing, for flagellates were found in only a small percentage of individuals and then only in scanty numbers. Surely if the bug was the true host, some indication of active multiplication, ending in a heavy infection, must have occurred. The bugs were all fed upon the thin red epidermis over the sore. Patton has stated that bugs so fed cannot possibly dislodge parasites from the sore, but that they only obtain them from the blood circulating at the spot, as their proboscis is not long enough to reach the parasites in the sore. I have shown elsewhere that this view is improbable, since the skin over the infected macrophages may be hardly more than 50 μ in thickness and that the proboscis of a bug could readily penetrate so far. I have now obtained a better proof of this, for in the case of fleas feeding on the sore I have shown definitely that they take up free parasites while feeding and these in numbers too great to admit of their having come merely from the circulating blood. If the flea can do this, why not the bug? The further details of the flea experiments will be found below. The following experiments were conducted with *Cimex lectularius* :—

Number of bugs fed.	Number of days between feeding and dissection.	Number of bugs giving positive result.
26	9	0
14	8	0
29	7	0
10	6	0
12	4-6	0
5	3	1
9	2	2
Total..... 105		3

All these were young bugs which had their first feed upon the sore, except seven which were dissected after eight days and eleven of the twelve which were dissected after four to six days. It will be clear from the table that of the one hundred and five bugs which fed on the sore only three gave a positive result. In these three the number of flagellates was small, so that it is just possible that some of the others were infected and the flagellate forms overlooked. A further point of interest is the fact that those infected were dissected only two or three days after feeding, while none of the much larger

number dissected later showed any such infection. If any active multiplication had taken place it is certain that I cannot have overlooked the infection. There is thus no evidence to show that the ingestion of a small number of leishmania will give rise to a massive infection. In my experiments conducted in Bagdad I found that one bug which was dissected twenty-four hours after feeding and three which were dissected forty-eight hours after, had become infected with flagellates. Patton found flagellate forms in bugs found upon the Cambay sore up to a much later date after feeding, and in one instance he claims to have found his post-flagellate forms in the bug.

The characters of the flagellates found in the *Cimex lectularius* do not differ in any way from those previously described by me. They resemble the forms met with in the artificial cultures. The flagellates usually occur in groups of three or four clustered together, indicating that such a group has probably developed from a single parasite. If the number of parasites ingested had been large as in Patton's Kala Azar experiments, then most certainly there would have been a corresponding number of rosettes of flagellates and one would have had a massive infection. It appears as if in the bug the development along the cultural lines proceeds to a certain extent and then comes to an end owing to failing nourishment; and just as in cultures which are old and in which the nutriment has been exhausted there appear round non-flagellate forms, so in the bug such forms are developed when the blood is nearing the completion of its digestion.

EXPERIMENTS CONDUCTED WITH FLEAS.

Owing to the fact that Basile claims to have transmitted the Mediterranean Infantile Kala Azar by means of fleas, and, further, that he has described flagellate forms from these which are regarded as developmental forms of leishmania, I thought it would be of interest to investigate the behaviour of *Leishmania tropica* in these animals. Basile, Sangiorgi, and Alvarez and da Silva claim that flagellate forms found by them in fleas taken from dogs infected with Kala Azar represent the leishmania taken up; Basile further claims that these developmental forms appear in the fleas when they are fed upon spleen-juice of infected dogs. It would seem, then, that the evidence in favour of these contentions was conclusive; but quite recently Nöller, who has conducted experiments in the transmission of *Trypanosoma lewisi* by means of the dog-flea *Ctenocephalus canis*, has shown that quite a high percentage (12 per cent.) of these fleas may harbour a natural herpetomonas which resembles so closely the developmental forms of leishmania that he concludes that the flagellates observed in the fleas by Basile and others are really natural flagellates and not leishmania as they have supposed. In his recent report on Kala Azar, Patton has also come to a similar conclusion; and from the experiments about to be recorded, I can testify to the great difficulty of such experiments owing to the possibility of a natural herpetomonas in these fleas. Unless one most carefully excludes such a flagellate, there is nothing whatever to prevent an error of interpretation, for the forms of herpetomonas of the flea so closely resemble the leishmania that microscopical examination is hardly sufficient to separate the two.

Nöller's Method of handling Fleas for Experiment.

In Nöller's most interesting paper on the transmission of *Trypanosoma lewisi* by the dog-flea, there is described a method of experimentation with fleas which has rendered easy the handling of these elusive and active creatures. I have employed this method and find it most suitable and convenient; and for the sake of those who may wish to experiment with fleas and have no opportunity of consulting Nöller's original article, I will describe the method, which is really the securing of the fleas on fine wire, after the manner of those who exhibit acrobatic and so-called tame fleas at Fairs and similar Shows. Nöller recommends the use of fine silver wire, 0.15 mm. thick. I have used the finest copper wire from a galvanic coil and find it works equally well. A piece of this, about 10 cm. in length, is doubled into a loop and the two ends are held between the finger and thumb of the right hand while the loop is held over a needle so that the wire is kept tight. By movement of the finger and thumb the wire is twisted till only a very fine loop is left at the end through which the needle passes. The exact size of the loop is rather an important matter, but it varies with the size of the flea to be used. Experience alone can act as a guide. The flea to be secured—and for first practice the large dog-flea is most convenient—is dropped on to the surface of water in a dish. It is then picked out with the finger and thumb of the left hand and held in such a way by the abdomen that the head is directed upwards and its dorsum towards oneself. By gently rolling the finger and thumb the head of the flea can be made to protrude while the abdomen is still held. The loop of wire is then taken in the right hand and carefully slipped over the flea's head. If the loop is of the correct size it will pass easily over the head but not back on to the abdomen. It will be seen that the flea commences to make violent struggles and finally it will manage to get its first pair of legs through the loop. Now is the critical moment. The flea is held only by the wire and with a fine pair of forceps the loop is pinched up smaller behind the dorsum of the flea. If secured in this way the flea cannot escape and will live quite well in cotton-wool for over two months. I have kept one flea alive in this way for two months and a quarter, and it was an unfortunate neglect of feeding that led to its untimely death. Those who have to use fleas in experimental work are much indebted to Nöller for introducing this simple method of handling them. With the wire bent in a suitable manner in two planes it will rest on the skin at any spot, and the flea will feed apparently in perfect comfort.

The Feeding of the Fleas.

Nöller has described the feeding of these fleas. After sucking blood for about ten minutes—it may be more or less than this—fæces are passed, and usually these are ejected with considerable force, so much so that they may fall on the skin as much as an inch away from the flea. The first fæces passed consists of digested blood, but even with this first fæces there is admixture with fresh blood, as is shown by the fact that live *Trypanosoma lewisi* may be passed in the first fæces when the flea is feeding on an infected animal. After the passage of fæces the fleas continue feeding and may pass fæces which eventually becomes pure blood as frequently as every half-minute. If the flea is observed by transmitted light with a hand-lens during the feeding act, it is noticed that the gut is in a state of most violent

peristaltic contractions, first in one direction and then in the other, so that the whole contents of the intestine are mixed together. It is this fact that accounts for the appearance of fresh blood in the first fæces passed.

It is a simple matter while the flea is feeding to collect the ejected fæces on cover-glasses held in forceps in a suitable position to receive them. The fæces can be made into films and stained. In this way it is easy to determine if a flea is infected with flagellates or not. The peristaltic action is so violent that if flagellates are present, and even if they are attached to the lining of the gut, some at any rate would be expelled. Thus in conducting experiments with leishmania it can readily be determined if a flea is clean by feeding it upon some uninfected person two or three times, and examining the fæces passed during these feeds. From a practical experience of this method I can testify to its accuracy, for those fleas which have been found naturally infected with herpetomonas or *Trypanosoma lewisi* have remained infected during the whole time that they have lived and fed upon human blood, while none of those which were not infected at the first feed have acquired any such infection unless fed upon infected blood.

*The Fæces passed by Fleas when Feeding on the Sore may
contain Leishmania.*

At the outset of my experiments with fleas and *Leishmania tropica* I found that fleas while feeding upon the sore passed fæces containing these parasites. It was evident that the fleas had taken them up directly from the sore and had dislodged them from the infected macrophages. The parasites are always free and not included in leucocytes, as they would be if they came from the peripheral circulation. I have noted the presence of these leishmania in the fæces in nearly every instance (upwards of 50 feeds) that a flea has fed upon the sore, so that there is no possibility of a confusion with any herpetomonas of the flea. The fleas, moreover, had given negative results when fed upon uninfected persons. It can thus be fairly safely concluded that in almost every instance that a flea fed upon this particular Oriental Sore—which, as I have said, was one peculiarly suited to such experiments—leishmania were taken up, as proved by examination of the fæces passed during the act of feeding.

The Leishmania do not Develop in the Flea.

In order to determine if any development would take place, the following procedure was adopted:—The clean flea was fed upon the sore and its fæces examined for leishmania. It was then kept in the cool incubator (22°–23° C.) for a variable time. It was then fed again, either upon the sore or upon my arm below the sore. The fæces were collected again and examined. When the feed was upon the sore, leishmania were again found to have been taken up, whereas in no case were they seen when the feed was on the skin below the sore, however near the sore that might be. So that the evidence is again in favour of the fleas having dislodged the parasites from the sore. This process of feeding on the sore at varying intervals, with the examination of the fæces passed during the feed, would give some indication of flagellates in the gut if any development of the leishmania had taken place. In fleas naturally infected with herpetomonas or *Trypanosoma lewisi* there is no difficulty in finding these in the fæces. Yet with the leishmania I never on any occasion found the least indication of a development into flagellates,

for the leishmania taken up at one feed had completely disappeared before the next. In a certain number of instances these observations were controlled by dissection of the fleas, but this gave no better result. So that I have obtained no evidence that *Leishmania tropica* will develop in the dog or human flea (*C. canis* and *P. irritans*), both of which were used.

Description of Experiments.

1. A *Ctenocephalus canis* was fed upon the sore twelve times and once upon the skin near it between July 29 and August 12. On nearly every occasion when it fed from the sore, leishmania were found in the fæces. The intervals between the feeds varied from one to eight days, and on no occasion was any development noted. On September 15 the flea was fed on a rat infected with *Trypanosoma lewisi* and again on a clean rat on Sept. 21, 24, and 26. On none of these occasions were developmental forms of *T. lewisi* seen in the fæces. On Sept. 27 it was again fed on the *T. lewisi* rat. On Oct. 3 it fed on the clean rat and the fæces contained the typical small trypanosomes which are characteristic of the *T. lewisi* infection of the flea. On Oct. 8 the flea was found dead. Though it did not become infected with leishmania, it became infected with *T. lewisi*.

2. A *C. canis* was fed on the sore six times and once on the skin near the sore between Aug. 3 and 31, when it was dissected. No indication of development was found either in the fæces or on dissection.

3. A *Pulex irritans* was fed upon the sore on Aug. 3 and 10. It was dissected with negative result on Aug. 13.

4. A *C. canis* was fed upon the sore on Aug. 10, 13, and 16. Fæces always negative as regards development. Dissected on Aug. 19 with negative result.

5. A *C. canis* fed on sore on Aug. 19 was dissected on Aug. 26—negative.

6. A *C. canis* was fed on sore on Aug. 24 and dissected Aug. 26—negative.

7. A *C. canis* fed on skin below sore on Sept. 6—fæces negative. On Sept. 12 fed on sore and on Sept. 15 on the *T. lewisi* rat. On both these occasions fæces contained no developmental forms of leishmania. On Sept. 21 flea fed on my arm—fæces negative; and again on Sept. 24 it had a small feed. On Sept. 25 flea fed on my arm and passed fæces containing typical small *T. lewisi* trypanosomes. It again fed on human blood on Sept. 28 and Oct. 1; on both occasions small trypanosomes were in the fæces. On Oct. 4 flea fed on a clean rat, while the fæces passed were collected on rat's hair and allowed to dry for one hour. It was then placed in mouth of another rat which did not become infected with *T. lewisi*. On Oct. 9 flea died and was found to have large infection of small trypanosomes in hind gut. Some of the fæces which were passed on Sept. 28 were placed without drying into the mouth of a clean rat. The rat was infected with *T. lewisi* six days later. This is a confirmation of Nöller's results. The clean rat upon which this flea fed was the same as that upon which the first flea fed after it had taken up *T. lewisi*. It was not contaminated with flea fæces and has not become infected.

8. A *C. canis* was fed on the skin of my arm on Sept. 2 and on the sore on Sept. 6. Fæces negative. Flea was dead on Sept. 12.

9. A *P. irritans* was fed on the *T. lewisi* rat on Sept. 21 and dissected on Sept. 25. It was found to contain the developmental forms of *T. lewisi*, so

that this flea, as well as the dog-flea, will probably prove to be a transmitter of *T. lewisi*.

10. A *C. canis* was fed on the sore on Oct. 3, 4, 8, 14, 21. On no occasion was there any developmental form in the fæces.

11. A *C. canis* was found, when fed on clean human blood, to be infected with the small *T. lewisi* trypanosomes. It must have previously fed on an infected rat. It was fed on the sore on Oct. 8 and Oct. 14. In fæces only unaltered leishmania and small *T. lewisi* trypanosomes.

12. A *P. irritans* was found to be infected with herpetomonas* at its first feed. From Aug. 24 to Nov. 4, when it died, it had had sixteen feeds of human blood and had constantly passed large numbers of herpetomonas, both flagellate and non-flagellate, in its fæces. These resemble very closely developmental forms of leishmania.

With the fæces of this flea collected on sterile cover-glasses cultures have been obtained on NNN medium as follows :—

1. Fresh fæces very rich culture.
2. Fæces allowed to dry for one hour at laboratory temperature in sterile Petri dish gave a culture after a few days.
3. Fæces dried for four hours gave only a growth of sarcinæ.
4. Fæces dried for two hours and a half gave growth of flagellates.
5. Fæces spread on cover-glass and dried for 24 hours gave culture of flagellates.

It is thus seen that the cystic forms passed in the fæces are very resistant and will stand a considerable amount of drying. Further, the fæces passed by the flea are, as a rule, sterile as regards bacteria which will grow upon the NNN medium. The cultures of herpetomonas are exceedingly vigorous and have now been maintained for several generations, so that they will probably prove to be culturable for an indefinite period.

13. A *C. canis* from a cat was found to be infected with herpetomonas on its first feed.

Conclusions drawn from these Experiments.

It is seen from these experiments :—

1. That the dog and human fleas, *Ctenocephalus canis* and *Pulex irritans*, readily take up *Leishmania tropica* from the sore and that some of them are passed in the fæces voided during the feeding act.
2. That if a flea is infected with flagellates the fæces voided during the feed will contain some of these.
3. That the leishmania taken up do not develop into flagellate forms to produce an infection of the flea's gut.
4. The fleas that have not become infected with leishmania may be infected with *T. lewisi*.
5. When once infected with herpetomonas or *Trypanosoma lewisi* the fleas remain so infected for long periods whether they feed upon human or rat's blood. The human blood appears to be quite favourable to the development of *Trypanosoma lewisi* in the flea.

* This flagellate of the human flea is quite distinct from the crithidium described by Porter from this flea. It is a true herpetomonas and has never shown any crithidial forms. After its death the flea was dissected and was found to contain enormous numbers of herpetomonas in all stages of development. (A. Porter, 'Parasitology,' 1911, pp. 237-254.)

6. *Trypanosoma lewisi* appears to undergo its development in the *Pulex irritans* as well as in the dog-flea *Ctenocephalus canis*.
7. As Nöller has shown, it is the faeces passed by the flea which, when licked up by the rat, produce an infection. The act of feeding itself apparently does not infect. This has been confirmed by feeding the dog-flea on a *T. lewisi* rat, feeding it in future on a second rat, and giving the faeces passed during the feed to a third rat. It is only the third rat that becomes infected. The incubation period was six days in the rat.
8. The small oval encysted forms of the herpetomonas passed by the flea are very resistant structures. They will withstand drying for 24 hours, and then give rise to a culture of herpetomonas when introduced into NNN medium.

The Bearing of these Results on Basile's Experiments.

The failure to obtain any development of *Leishmania tropica* in the dog or human flea and the fact that the flea may contain a natural herpetomonas almost indistinguishable from cultures of leishmania suggest to my mind that Basile and those who have followed him may have misinterpreted what they found in the fleas. Basile gave no proof that he had taken care to exclude a natural flea infection, and, as a matter of fact, the number of fleas found by him to be infected with what he interpreted as leishmania was well below the percentage found in other places to be naturally infected with herpetomonas. Comparing the herpetomonas found by me in the fleas I find that they correspond entirely with those described by Basile and Alvarez and Pereira da Silva.

It may be urged that my flea experiments were conducted with *Leishmania tropica* and Basile's with the leishmania of Infantile Kala Azar and that the results are not comparable. The leishmania derived from all sources behave so similarly in culture media and in bed-bugs that if the one develops to any extent in such a host as the flea it would be expected that the other would at least show some indication of such a development. Still I recognise this difference, and only trust that now that Nöller has shown us how fleas can be conveniently handled for accurate experiment, Basile or some other observer will repeat the experiments with every precaution to exclude any error.

I make no comment on the actual transmission experiments carried out by Basile in transmitting Kala Azar to dogs by means of fleas*. Experiments conducted in the Sudan by Marshall with infected dogs and fleas have given only negative results. I am quite in accord with Nicolle and Basile, who maintain that the Kala Azar of the Mediterranean districts is the same disease in children and dogs. Patton finds himself in complete agreement

* Quite recently, in the 'Bulletin de la Société de Pathologie Exotique' of Oct. 9, 1912, the brothers Sergeant, Lhéritier, and Lemaire have described the successful transmission of Canine Kala Azar from dog to dog by means of the flea *Ctenocephalus canis*. It is unfortunate, from the point of view of the experiment, that the dog which was found to have become infected never showed any parasites in its liver, for it was by liver puncture performed before the experiment that the dog was supposed to be free from infection. After infection was proved by examination of the spleen and bone-marrow, both microscopically and by culture, the liver still gave negative results. Flagellates were found in the fleas taken from the infected dog and also in others from uninfected animals, but the authors have not proved that they were developed from leishmania taken up by the fleas.

with Gabbi, who regards the infantile disease as distinct from the canine. It seems to me that the established fact that in all the endemic centres for Infantile Kala Azar along the Mediterranean there canine Kala Azar exists, is in itself almost convincing. There are such cases as that recorded by the Sergents, Lombard, and Quilichini, where a child, a dog, and a cat were found to be infected with Kala Azar in an isolated farm near Algiers, which are difficult to explain on any other hypothesis. In addition we have the experimental evidence of the possibility of producing Kala Azar in dogs by inoculation of the human virus. Surely it is a greater assumption to conclude under these circumstances that the canine disease is distinct from the human than that they are one.

The distribution of the Mediterranean Kala Azar in children and dogs points to the flea as a likely transmitter of the malady. Basile claims to have proved this, but Franchini's recent results, in which he has found that the leishmania taken up from spleen puncture material develop into flagellates in these insects' guts, opens another possibility, for mosquitoes as well as fleas feed upon both dog and man. In India it is quite possible that the bed-bug is the carrier of the disease. It is an insect which has been blamed for the transmission of many maladies, but has been clearly proved to transmit none. At present it is still unconvicted, for I fail to find in the fact that the leishmania will develop in its gut even to Patton's post-flagellate stages any conclusive evidence that it is the true host and natural bearer of this fatal malady. Till the experiment of transmission has been carried out we must be content to keep an open mind upon this subject.

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